universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Essential oils as „a cry for help“: a review

verfasst von

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angestrebter akademischer Grad

Magistra der Pharmazie (Mag.pharm.)

Wien, 2014

Studienkennzahl lt. Studienblatt:	A 449
Studienrichtung lt. Studienblatt:	Diplomstudium Pharmazie
Betreuer:	Univ. Prof. Mag. Pharm. Dr. Gerhard Buchbauer

Danksagung

Ich möchte mich an dieser Stelle sehr herzlich bei Herrn Univ. Prof. Dr. Gerhard Buchbauer für die Beratung und Betreuung während des Verfassens meiner Diplomarbeit bedanken.

Meinen Eltern, die mir das Pharmaziestudium ermöglicht und mich in allem unterstützt haben, möchte ich ganz besonders danken. Ich bin meiner Familie sehr dankbar, dass sie immer für mich da war, wenn ich sie gebraucht habe.

Auch meinem Partner Jürgen Strommer will ich Danke sagen für seine Hilfe bei jeder computertechnischen Frage und für seine Fähigkeit mich immer wieder zum Lachen zu bringen.

Abstract

This work is an update of a recently published review and is consistently referred to this article and recent findings about plants' indirect defence are added on. Herbivore induced plant volatiles (HIPVs) and their effects on the third trophic level that involves predators and parasitoids are discussed in this review. The fact that plants are not passive individuals is confirmed on the basis of several studies. Plants can perceive and respond to cues in their environments with plastic morphological, physiological and behavioural traits. Plasticity allows plants to tailor their defences to their current and expected risks caused by herbivores. The "cry for help" of plants is also observed from the carnivores' point of view. The volatile mixture contains crucial information for decisions of carnivorous insects. Furthermore, the most important methods to examine the behavioural response of carnivorous insects to HIPVs are presented not only in laboratory set ups but also in the field. Manipulations of plants by silencing genes or overexpressing genes can help to understand mechanisms of indirect defence. Various interesting examples of indirect defence reveal the possibility to use HIPVs in biological control. Therefore, the application of synthetic pesticides, that pollute the environment, may be reduced in the future.

Zusammenfassung

Dieser Bericht ist ein Update einer kürzlich veröffentlichten Arbeit. Es wird immer wieder Bezug auf diese Arbeit genommen und die neuesten Erkenntnisse über indirekte Abwehrmechanismen der Pflanzen werden hinzugefügt. Herbivor-induzierte Pflanzenduftstoffe (HIPVs) und ihre Wirkungen auf die dritte trophische Ebene, welche Räuber und Parasitoide umfasst, werden in diesem Bericht behandelt. Die Tatsache, dass Pflanzen keine passiven Individuen sind, wird anhand mehrerer Studien bestätigt. Pflanzen können Signale in ihrer Umwelt wahrnehmen und auf diese mittels ihrer morphologischen, physiologischen und verhaltensbezogenen Plastizität reagieren. Plastizität erlaubt es Pflanzen ihre Abwehrkräfte auf momentane und bevorstehende Gefahren, die von Herbivoren ausgehen, anzupassen. Der „Hilfeschrei“ der Pflanzen wird auch aus der Sicht der carnivoren Insekten betrachtet, welche entscheidende Informationen durch die komplexen flüchtigen Mischungen erhalten. Außerdem werden die wichtigsten Methoden präsentiert, mit denen man das Verhalten der Carnivoren auf HIPVs nicht nur im Labor, sondern auch im Freiland untersuchen kann. Manipulationen der Pflanzen durch Ausschaltung oder übermäßige Expression von Genen können dabei helfen, Mechanismen der indirekten Abwehr zu verstehen. Verschiedene Beispiele der indirekten Pflanzenabwehr zeigen, dass HIPVs in der biologischen Schädlingsbekämpfung eingesetzt werden könnten. Dadurch kann möglicherweise der Einsatz von synthetischen Pestiziden, welche die Umwelt stark belasten, in Zukunft reduziert werden.

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1. INTRODUCTION

Plants are a favoured food not only by humans. Five thousand species of mammals and more than three million insect species belong to the group of herbivores. Plants stand at the beginning of the food chain and enable the living of animals and humans. Therefore, it is important that plants can be also defensive and inedible, otherwise they would have been devoured long ago and would have caused the downfall of wildlife and human beings. The coexistence of plants and animals would not be possible without an effective plant self-defence. Thereby the plant's scope seems to be rather limited because they are deep-rooted, rarely moveable and light-dependent organisms. However, they have developed surprisingly visionary and inventive defence strategies. [1]

Plant volatiles are used to attract pollinators and seed dispersers. In addition, volatiles are involved in further two physiological processes, namely plant-plant interaction and the attraction of symbiotic organisms. Plants advertise for the services of parasitoids and predatory arthropods in the surrounding community to get their herbivorous enemies off. [2] Carnivorous insects take advantage of the fact that, after herbivory, many plants emit volatile mixtures which differ in both quantity and composition from those emitted before herbivory. [3]

Moreover, plants have to react to an attack in an appropriate way. Induced defence reactions depend on degree and type of damage. For example, continuous damage caused by herbivores needs more intensive defences than a single damage mechanically caused. The skill of plant defence consists not therein to deter as much enemies as possible and that for any price. The strived target is to soak this price adequately. The best defence is useless when the whole resources are devoured abruptly and afterwards no energy is left for growth and formation of flowers and seeds. [1]

Great amounts of synthetic herbicides have been extensively used for the control of pests during the past few decades. Since the application of these herbicides cause health, environmental and toxicological problems, the ambition is high to change to natural plant products, like plant volatiles, which are safer and ecologically friendly. [4]

2. BASIC INFORMATION

2.1. The underlying mechanisms of indirect defence

Oxylipins are an important class of VOCs. They originate from polyunsaturated fatty acids released from chloroplast membranes by lipase activity, and represent the precursors of many oxygenated compounds, including jasmonates (jasmonic acid [JA], jasmonic acid methyl ester [JAMe], amino acid conjugates and further metabolites of JA) and green leaf volatiles (GLVs). [2]

Octadecanoids are central compounds in the defensive responses when plants are attacked by insects, mites or microorganisms. [5] The cascade is initiated by the mechanical wounding of cellular membranes, for example by the caterpillar feeding. Thereby α-linolenic acid (18:3) is released from the membranes. Lipoxygenases (LOX) form hydroperoxides from linoleic acid (18:2) or α-linolenic acid (18:3) in plastids (Figure 1). [2] Linoleic acid as the substrate is converted into (13S)-hydroperoxyoctadecadienoic acid (13-HPOD) and (9S)-hydroperoxyoctadecadienoic acid (9-HPOD), whereas linolenic acid as the substrate is converted into (13S)-hydroperoxyoctadecatrienoic acid (13-HPOT) or (9S)-hydroperoxyoctadecatrienoic acid (9-HPOT). Discrete 9-LOX and 13-LOX pathways have been proposed to explain the occurrence of numerous oxylipins. The enzyme 9-allene oxide synthase (9-AOS) leads to the synthesis of an epoxy intermediate that yields 10-oxophytodienoic acid, whereas 13-allene oxide synthase (13-AOS) activity forms precursors for the synthesis of cis-(+)-12-oxo-phytodienoic acid (12-OPDA). [2] 12-OPDA is transferred from the plastid into the peroxisome, reduced and its chain is shortened by β-oxidation to yield jasmonic acid (3R,7S stereoisomer of JA).[5] Both 12-OPDA and the oxylipins may interact with their molecular target and cross-talk to other signalling pathways. [5]

The SCF complex is an abbreviation of its components: the **S**-phase kinase-associated protein 1 (Skp1), the **C**ullin frame protein (Cul1), the **F**-box protein and the RING-box protein 1 (Rbx1):[6]

- Skp1: This protein forms part of the horseshoe-shaped complex, together with the Cul1 protein. Skp1 is essential in the recognition and binding of

the F-box.[7]

- Cul1: This protein generates the major structural frame of the SCF complex. It connects the Skp1 domain with the Rbx1 domain. [6]

- F-box: The name F-box is derived from cyclin F, in which it has been first identified. F-box is responsible for the specificity of SCF by aggregating to target proteins independently of the complex and then binding to the Skp1 component. Thus the protein is brought into proximity with the functional ubiquitin-conjugating enzyme (E2 protein). [6]

- Rbx1: This component contains a small zinc-binding domain called the "RING Finger", to which the E2-ubiquitin conjugate binds. Thereby the transferal of the ubiquitin to a lysine residue on the target protein is enabled. [6]

JA is transported to the cytosol where it is conjugated specifically to isoleucine (Ile) by an enzyme. JA-Ile synthesized in the cytosol presumably diffuses into the nucleus where it stimulates binding of jasmonate ZIM-domain (JAZ; ZIM protein is derived from an Arabidopsis gene, the so-called "zinc finger protein expressed in inflorescence meristem"-gene[5]) to the gene Coronatine Insensitive 1 (COI1) to activate gene expression. [8]

Many aspects of eukaryotic development depend on regulated protein degradation by the ubiquitin-proteasome pathway. This highly conserved pathway promotes covalent attachment of ubiquitin to protein substrates through the sequential action of three enzymes called a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). Most ubiquitinated proteins are then targeted for degradation by the 26*S* proteasome. [9] SCF is an E3 ubiquitin ligase. [8]

COI1 is a F-box protein component of the E3 ubiquitin ligase (SCF^{COI1}) and it functions as the substrate-recruiting module. Recent studies have identified the JAZ-domain family of transcriptional repressors as the SCF^{COI1} substrate targets, which associate with COI1 in a hormone-dependent manner. [8]

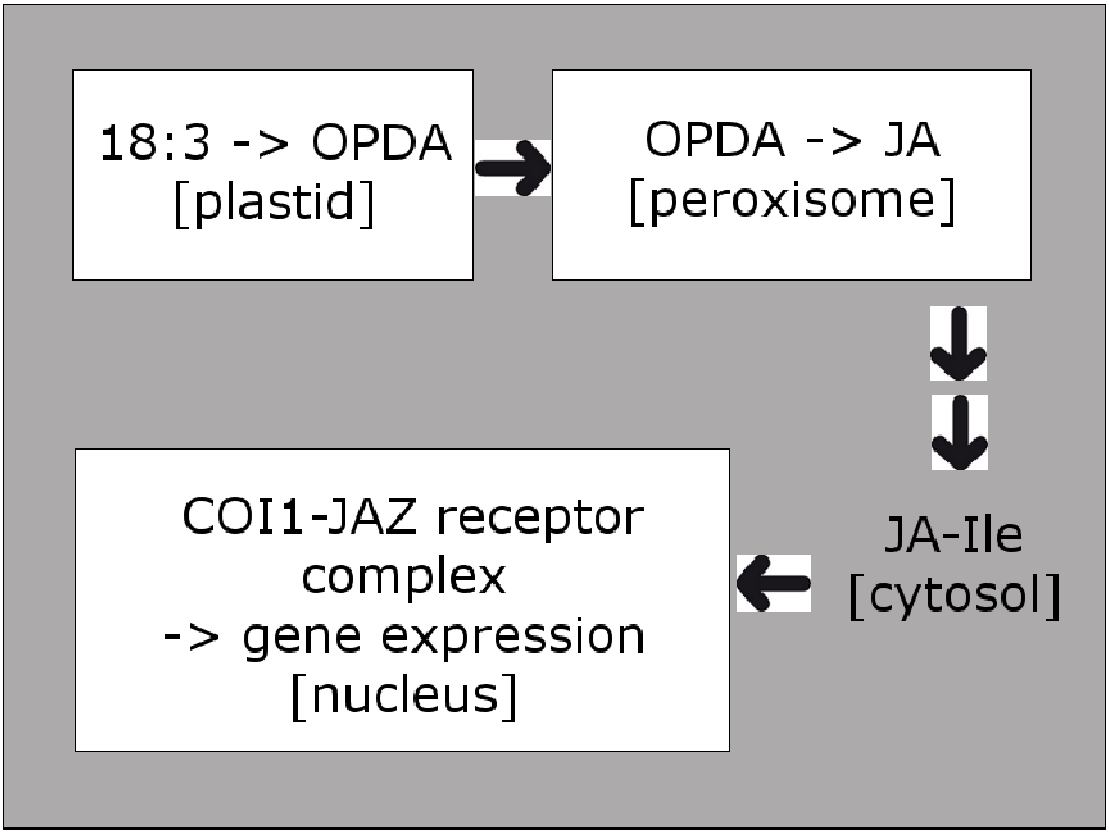


Figure 1: JA biosynthesis and activation

Methylation of JA by a specific methyl transferase produces JAMe. Constitutive over-expression of the JA-specific methyl transferase leads to a higher amount of JAMe, an unchanged JA level, and increased pathogen resistance, indicating that JAMe can be an active form of JA under specific conditions. [2]

The essential oil of jasmine comprises more than a hundred components, but the most important contributions come from cis-jasmone. This compound has recently gained additional attention due to its production from herbivore-damaged leaves. [2]

Natural elicitors of indirect defence are JA and coronatine. The active form of the plant hormone is the conjugate of the (3R, 7S)-stereoisomer of JA with L-isoleucine. The required stereoisomer rapidly epimerises to the less active (3R, 7R)-stereoisomer (Figure 2) which may serve as a mechanism for signal attenuation to avoid an exaggerated response. The biological effect of JA is also mimicked by coronatine (Figure 2), a bacterial phytotoxin originally described in *Pseudomonas syringae*. [8]

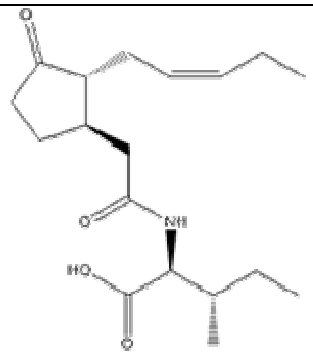
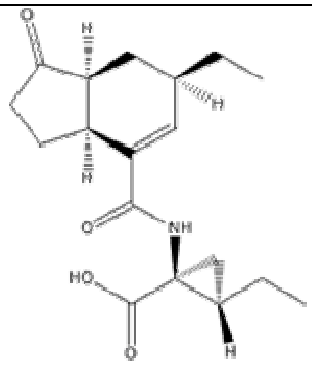
Structural analogues	
	
jasmonoyl-isoleucine conjugate	coronatine

Figure 2: Natural elicitors of plant defensive responses. [5]

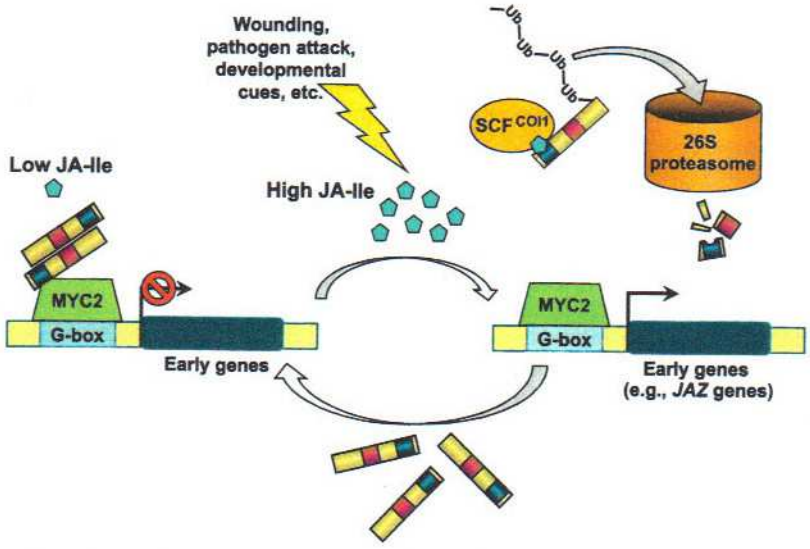


Figure 3: Jasmonate signaling. [10]

The basic-helix-loop-helix (bHLH) transcription factor MYC2 (AtMYC2; MYC2 is the abbreviation of Mouse-ear cress, which is a synonym of Arabidopsis thaliana) regulates the JA signaling pathway in Arabidopsis. JAs are plant hormones, which are essential in plant defence and development. MYC2 coordinates JA-mediated defence responses by antagonistically regulating two different branches of the JA signaling pathway that determine resistance to pests and pathogens, respectively. MYC2 is required for induced systemic resistance (ISR) triggered by beneficial soil microbes while MYC2 function is targeted on pathogens during effector-mediated suppression of innate immunity in roots. Another function of MYC2 is the regulation of crosstalk between the

signaling pathways of JA and those of other phytohormones such as abscisic acid (ABA), salicylic acid (SA), gibberellins (GAs) and auxin/indole-3-acetic acid (IAA). MYC2 also regulates interactions between JA signaling and light, phytochrome signaling and the circadian clock. MYC2 is involved in JA-regulated plant development, lateral and adventitious root formation, flowering time and shade avoidance syndrome. Related bHLH transcription factors MYC3 and MYC4 also regulate both overlapping and distinct MYC2-regulated functions in *Arabidopsis.* Furthermore, MYC2 orthologs can regulate JA-mediated biosynthesis of secondary metabolites. [11]

The core jasmonate (JA) signaling module is COI1-JAZ-MYC2. This modul allows a full view of the JA signaling pathway from hormone perception to transcriptional reprogramming. JAZ proteins are repressors of MYC2 and targets of SCF^{COI1}, which is the likely jasmonate receptor. Upon hormone perception, JAZ repressors are degraded by the proteasome. Thereby MYC2 is released and the activation of JA responses is permitted. All members of the JAZ family share two conserved domains, the Jas motif, required for JAZ interactions with MYC2 and COI1, and the ZIM domain, the function of which is so far unknown. The ZIM domain acts as a protein-protein interaction domain mediating homo- and heteromeric interactions between JAZ proteins. [12]

Low levels of JA-Ile permit the accumulation of JAZ proteins that repress the activity of the transcription factor MYC2 and likely other transcription as well. In response to developmental or environmental cues JA synthesis is activated. High levels of JA-Ile promote SCF^{COI1}-mediated ubiquitination and subsequent degradation of JAZ proteins via the 26S proteasome. Following its release from JAZ-mediated repression, MYC2 positively regulates the expression of primary JA-responsive genes, including JAZ genes, which contain a G-box in the promoter region. Newly synthesized JAZ repressors presumably establish a negative feedback by binding MYC2 and attenuating expression of JA-responsive genes. These JAZ complexes may be the functional unit for MYC2 repression. [10]

Attraction of parasitoids or predators by HIPVs has been well-demonstrated in many plant species both in the laboratory and in the field. With regard to the

underlying mechanisms, it has been demonstrated that the octadecanoid pathway, with the plant hormone JA as central component, plays an important role in regulating HIPV emission, although the shikimic acid and ethylene pathways can play roles as well. For example, in Lima bean plants, a transient increase of endogenous JA in leaves is involved in the induced synthesis of HIPVs, and application of exogenous JA to leaves leads to the induction of a volatile blend similar to the HIPV blend induced by spider mites. Conversely, blocking JA synthesis or its action results in the reduction of volatile emission, and consequently interferes with the attraction of predators to herbivore-damaged plants. However, only a few studies on indirect plant defence have considered plants attacked by multiple herbivore species, whereas this is a widespread phenomenon in nature. Cross-talk between different defence signaling pathways has important consequences for the evolution of plant defence, as it can influence the amount of damage suffered by plants and subsequently influence selection pressure on defense response. [13]

2.2. General view of plant volatiles

Volatile blends of plants are complex and the mixtures differ among the plant parts. They are as complex as the roles they play in the live of a plant. [1]

Methanol and ethylene are two of the most frequently emitted compounds among the one- and two-carbon plant volatiles included in the volatile blends. Methanol is released in part from the demethylation of the abundant cell-wall constituent pectin when leaves change shape during growth or senescence, or when they are attacked by herbivores whose oral secretions have a high pH. The pH shift at the feeding site that occurs when such larvae consume leaves activates pectin methylesterases (Figure 4) in the cell wall. This enzyme releases copious quantities of methanol. Ethylene is one of three plant hormones that are emitted into the air in biologically active quantities and is derived from the oxidation of 1-amino-cyclopropane-1-carboxylic acid (Figure 4), which in turn is derived from the amino acid methionine.

Ethylene is the only plant volatile for which the molecular mechanisms of perception (by the ethylene receptor) are understood in detail (Figure 4). Knowledge of how ethylene is perceived has helped to explain its function.

When plants are transformed with a mutated version of this receptor (etr1-1), the resulting plants are essentially deaf to their own ethylene emissions and begin to release huge quantities of ethylene. This mechanism reveals the close interplay between production and perception. If the production and release of other plant volatiles turns out to be similarly regulated, it would become clear how the greatly amplified releases could have evolved, which are required for the defence functions of plant volatiles. These ethylene-deaf plants also lose their ability to sense the location of other nearby plants and physical objects in their surroundings. [1]

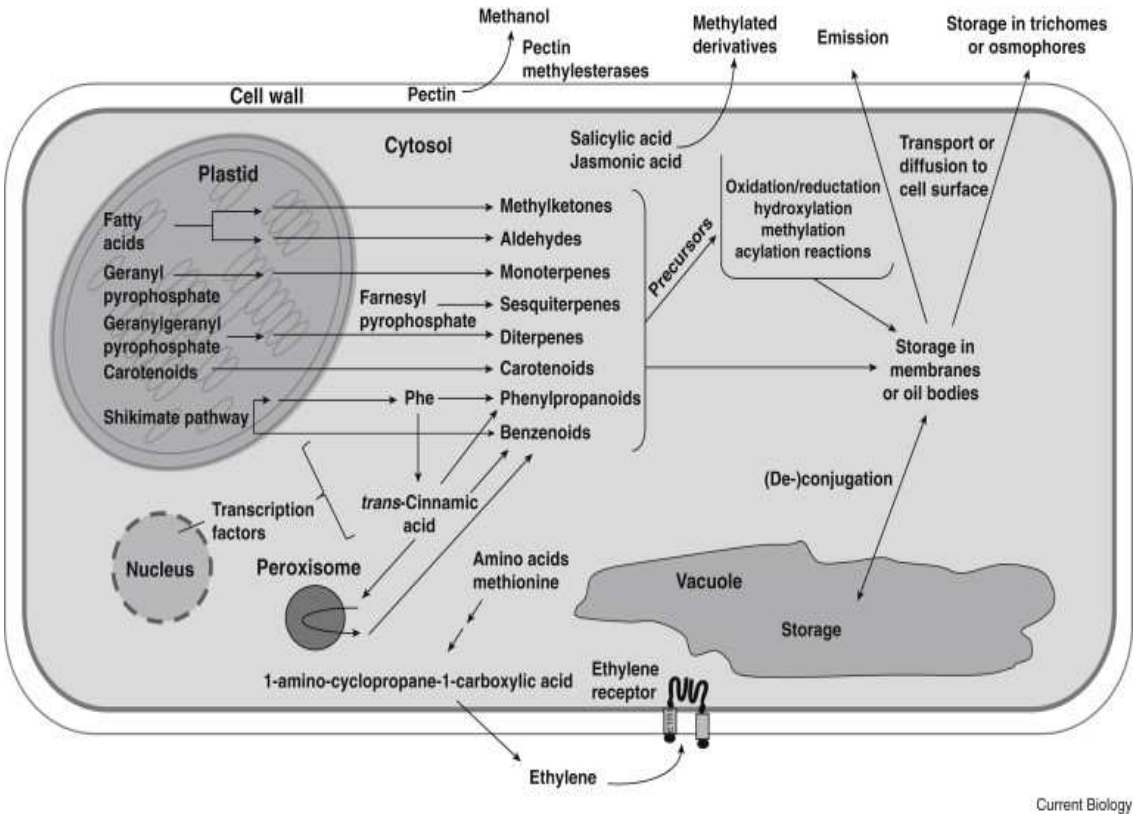


Figure 4: Origin of volatiles [1]

The constituents of volatile blends, which tend to be lipophilic, are frequently synthesized from hydrophilic precursors whose hydrophilic functional groups have been removed or masked through reduction, methylation and acylation reactions. Their lipophilicity allows plant volatiles to cross membranes easily and evaporate into the atmosphere. The volatile synthesis reactions are combinatorial. Thereby, constituents of many different biosynthetic pathways can contribute to the chemical diversity of the emitted bouquets. [1]

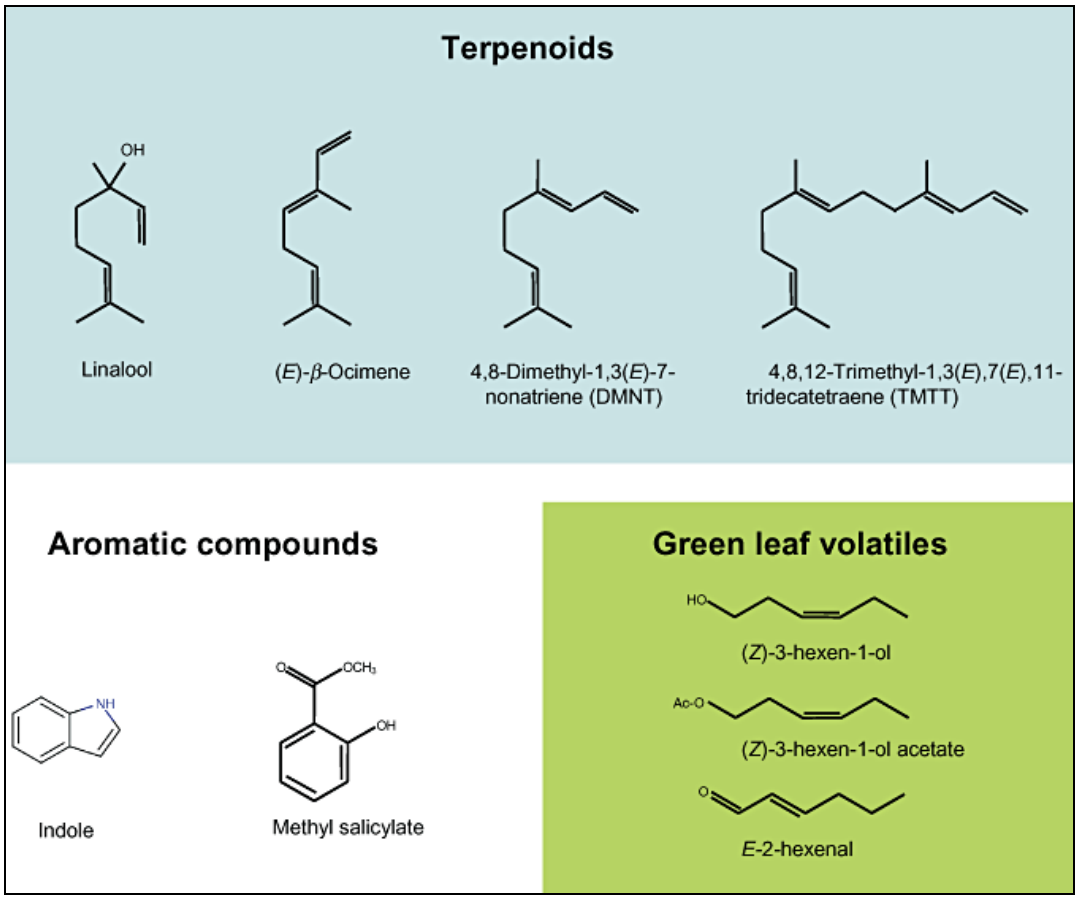


Figure 5: Plant volatiles [13]

Plants produce a large range of metabolites that are volatile because of their high vapor pressure under standard conditions.[3] Over 1700 volatile compounds have been identified from more than 90 plant families[2], but only a fraction of these are emitted by individual plants after herbivore damage. These can be grouped into four categories: [3]

Terpenes

The largest class of plant volatiles are terpenes or terpenoids which are classified by the number of branched C5 units in their structures. Major terpene volatiles emitted from vegetative tissue include the C5 compound isoprene (one C5 unit), monoterpenes, such as (*E*)-β-ocimene and linalool (two C5 units), and sesquiterpenes, such as (*E*)-β-caryophyllene and (*E*, *E*)-α–farnesene (three C5 units). Two terpenes that occur frequently after herbivore damage have irregular structures, the homoterpenes C11 (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and C16 (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).[3] All

isoprenoids are produced from the precursors dimethylallyl diphosphate (DMAPP) and its isomer isopentenyl diphosphate (IPP), which are synthesized by the methylerythritol phosphate (MEP) pathway in the chloroplasts and by the mevalonate (MVA) pathway in the cytoplasm. Some exchange and/or cooperation exists between these two pathways, that probably operate under different physiological conditions within the cell, and depend on the cell and plastid developmental state. The MEP pathway leads to the formation of monoterpenes and diterpenes. Diterpenes are precursors of the homoterpene TMTT and of the carotenoid-derived β-ionone. The hemiterpene isoprene is the simplest terpenoid emitted by plants and it is synthesized from DMAPP produced by the MEP pathway. Sesquiterpenoids are generated from farnesyl diphosphate (FPP), which comes from the cytosolic MVA pathway. The homoterpene DMNT derives from the sesquiterpene nerolidol. [2]

Generally, monoterpenes are typical leaf products whereas sesquiterpenes are typical flower fragrances, although the most common single compounds in floral scent are the monoterpenes limonene, (*E*)-β-ocimene, myrcene, linalool, α- and β-pinene. Large amounts of monoterpenes and sesquiterpenes are also produced in leaf glandular trichomes and are emitted from herbivore-damaged foliage and roots. [2]

The most typical compounds related to biotic stress are the homoterpenes 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) and 4,8-dimethylnona-l,3,7-triene (DMNT). TMTT might function as a signal as well as a phytoalexin that directly contributes to restricting bacterial growth in inoculated leaf tissue. [2]

Fatty acid derivatives

The oxidation of fatty acids leads to the formation of a large family of volatile derivatives, which are called GLVs due to the typical odour of green leaves.[3] After mechanic and herbivore damage GLVs are almost immediately released. GLVs are synthesized via the LOX pathway from C18 polyunsaturated fatty acids including linoleic and α-linolenic acids. The C18 acids are cleaved to C12 and C6 compounds by hydroperoxide lyases. The first C6 GLV compound (*Z*)-hex-3-enal is synthesized from the precursor 13-HPOT by the LOX/lyase

pathway. (*Z*)-Hex-3-enal is then converted to other GLVs, such as (*E*)-2-hexenal (leaf aldehyde) that leads to 2-hexenol, or to (*Z*)-3-hexenol (leaf alcohol) and (*Z*)-hex-3-enyl acetate (leaf ester). The latter is formed from a reaction between (*Z*)-3-hexenol and acetyl-CoA that is catalyzed by an acyltransferase.[2]

Aromatic compounds

The metabolism of phenylalanine leads to a group of compounds with simple aromatic rings and C1–C3 side chains, whereas a compound of tryptophan biosynthesis leads to indole derivatives. The most important representatives of this group after herbivore damage are methyl salicylate and indole (Figure 4).[3]

Salicylic acid (SA) is synthesized in plants by two pathways: one derived from benzoate via cinnamate, and the other via isochorismate. Methyl salicylate is synthesized in a reaction catalyzed by a methyltransferase whereby a methyl group is transferred from the donor molecule S-adenosinemethionine (SAM) to the carboxyl group of SA. Salicylic acid methyltransferase (SAMT) has been characterized in several plant species including the model plant Arabidopsis.[2]

In corn, indole is made by the cleavage of indole-3-glycerol phosphate (IGP), an intermediate in tryptophan biosynthesis. For example, indole has been identified as one constituent of the volatile blend which is emitted from corn in response to herbivore damage. The production and release of this compound in plants has been shown to be an active process in which *de novo* synthesis is triggered in response to insect feeding.[2]

Amino acid derivatives

After herbivore damage, various amines, oximes, nitriles, isothiocyanates and sulfides are released that are produced from amino acids. These compounds are often not as well recovered in standard headspace collections as terpenes, GLVs and aromatics. They may be more abundant than is currently realized. [3]

Differences are existing between the variable substance classes of plant volatiles relating to biosynthesis. GLVs are produced when leaves are damaged, independent of the agent causing the damage. Primarily GLVs are emitted from damaged leaf tissues. In contrast, monoterpenes, homoterpenes

and sesquiterpenes are produced in response to herbivore damage and generally released not only from damaged tissue but also from undamaged leaves and flowers. The homoterpenes TMTT and DMNT are the most characteristic compounds released after herbivore damage. The one- and two-carbon plant volatiles methanol and ethylene are probably not specific enough to indicate herbivore damage.[2]

2.3. Elicitors of VOC biosynthesis in plants

The elicitors that activate the release of volatiles after attack are frequently specific to the herbivore or to its feeding behaviour, as they are constituents of the herbivore's oral secretions. The saliva may contain fatty-acid–amino-acid conjugates, lytic enzymes (such as β-glucosidase) or fragments of plant ATPases, which are reintroduced into the wounds during feeding to elicit the production of new volatiles. Some herbivores insert their eggs into plant tissues, after which their oviposition fluids elicit changes in the volatiles released. [1]

In maize, increase in volatile production is due to larval elicitors like volicitin [N-(17-hydroxylinolenoyl)-L-glutamine] that are formed in the larval gut and introduced into the leaf during larval feeding. Volicitin appears to be relatively specific for the induction of defence in maize and other grasses because it does not induce volatiles in the dicot lima bean (*Phaseolus lunatus* Linne*,* Fabaceae). [4]

An important intermediate of the signal transduction pathways from herbivore damage to volatile production is JA in both maize and rice. The biosynthesis of jasmonate derivates in response to biotic stress has been best studied in dicot plants where JA, JAMe, amino acid conjugates of JA, and pathway intermediates like cis(+)-12-oxophytodienoic acid are formed and subsequently activate different parts of a complex signaling network. [4]

In *Nicotiana attenuate* Torr. ex S. Watson (Solanaceae), the JA-Ile conjugate is crucial for the induction of nicotine production after herbivore damage to the plant. [4]

In Arabidopsis (*Arabidopsis thaliana* Heynh, Brassicaceae), a complex of JA conjugate and COI protein targets a repressor of the JAZ family for degradation

by the 26S proteasome and thereby activates genes of plant defence. [4]

The WRKY domain is defined by the conserved amino acid sequence WRKYGQK at its N-terminal end, together with a novel zinc-finger-like motif.[14] The WRKY DNA-binding proteins belong to a large family of transcriptional regulators that has to date only been found in plants. Although their precise regulatory functions are largely unknown, the fact that these transcription factors appear to be specific to plants, with probably up to 100 members in *Arabidopsis*, suggests that they play an important role during plant evolution.[15] WRKY transcription factors probably are involved in plant defence. They regulate the jasmonate-dependent expression of a terpene synthase in cotton (*Gossypium hirsutum Linne*, *Malvaceae*), which is responsible for the production of volatile (+)-δ-cadinene.[4]

In grasses, especially rice and maize, the enzyme class of terpene synthases is responsible for the high number of volatile monoterpenes and sesquiterpenes. In rice, three herbivore-induced terpene synthases are sufficient to produce the majority of the terpene volatiles. The terpene blends of maize are formed by at least six sesquiterpene synthases. Three of these enzymes, TPS1, TPS10, and TPS23, are strongly induced by herbivore damage and produce the major sesquiterpene components of herbivore-induced volatiles. These terpene synthases can also be induced by JA treatment of the plant. [4]

Volatiles of maize plants were also shown to elicit responses in neighboring plants. This phenomenon is called priming. It involves increased transcription of defense-related genes and allows the plant to respond faster and more intensely to herbivore attack. [4]

2.4. Analytical devices to detect plant volatiles and responses of carnivorous insects

Olfactometer

An olfactometer is a device for detecting the response of an organism to odors. Air is passed over a potential stimulant and insects are induced to move upwind to the source. They have to make a choice or choices along the way to ascertain that the odour, not just air movement or moisture, is providing the

stimulus. Olfactometers traditionally have been Y-tube devices, in which moving insects chose between two sources of air flows that were blended together. Flight chambers accommodate flying insects in a similar manner, with air flowing over two or more sources of odor, but insects have not to traverse tubes. [16]

Electroantennogram detection

The electrical signal of an insect antenna can be recorded with an electroantennogram. This technique is employed for example to determine the volatiles and pheromones that can be perceived by an insect. Therefore, the antenna of a dazed insect is mostly prepared. The electroantennogram is recorded with a special apparatus. [17]

Inert gas flows into the chromatograph at a controlled flow rate. Gas flows into the chromatograph through the column, a thin tube coated with polymer. The column coil is heated to a controlled temperature. A sample of volatiles is injected into the injector input dock. After sample has traversed the column, it is split into two streams: one branch flows into the detector to produce a record of the retention times of the chemical mix (FID/flame ionization detector), and the other branch flows over a real insect antenna, which is wired with electrodes to record neural spiking activity (EAD/ electroantennogram detection). [17]

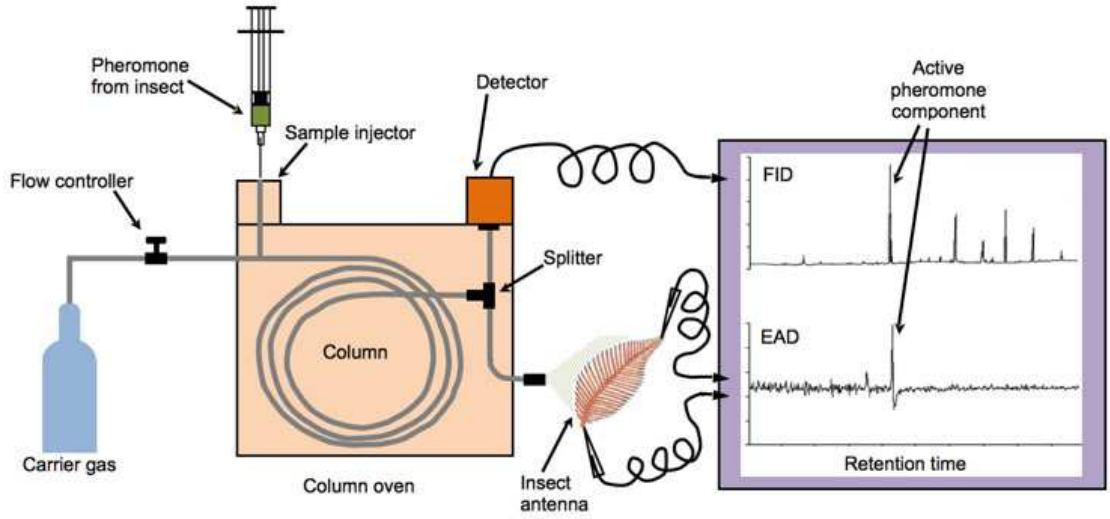


Figure 6: Gas chromatograph coupled with flame ionization detector and electroantennogram recorder [17]

The community of organisms that pay attention to plant volatiles are using biological receptors whose resolution and sensitivity are arranged at the very limit of the most advanced chemical techniques available. When such biological receptors are coupled to gas chromatography (GC) columns, as in GC–electroantennography, the best mass spectrometers can not compete. Although biological receptors are more sensitive than their mechanical counterparts, they are also more selective and may respond to only a single isomer of a constituent in a blend. In addition, analytical advances have also revealed the bias inherent in chemical detection systems. By extending the mass range and resolution, the newest analyses have revealed that a plant's metabolic status is encrypted in volatile bouquets, an insight which provides a new dimension for understanding the function of plant volatiles. [1]

Detection of plant volatile blends

Since plant volatiles are complex blends, they have to be measured with special apparatusses, such as gas chromatograph and mass spectrometer.

Floral bouquets are probably the best studied: to date more than 1700 different structures have been identified. This remarkable accomplishment is accredited to rapid developments in gas chromatography and mass spectrometry over the past four decades. [1]

Analysis of GLVs is difficult because of their chemical instability. Though the use of on-line techniques, like proton transfer reaction mass spectrometry (PTR-MS), allows their easy monitoring. [2]

A full characterization of the plant volatile blends is essential if one wants to understand their biological function. [1]

2.5. Some important herbivorous insects

Bean pod borer

Scientific name	*Maruca vitrata, M. testulalis, Crochiphora testulalis* **[18]**
Synonyms	Legume pod borer, Lima bean pod borer, Maruca **[18]**
Order	Lepidoptera **[18]**

Host plants	Beans and legumes [18]
Distribution	Asia, Europe, Africa [18]
Damage	The larval feeding causes round holes in the corolla of the flowers, distorted pods, webbed flowers and young pods, and presence of frass. Damaged flowers become a mass of brownish-frass a day after infestation[18]

Figure 7: Bean pod borer [19]

Tobacco hornworm

Scientific name	*Manduca spp.* [20]
Synonym	Tomato hornworm, Tobacco hornworm [20]
Order	Lepidoptera [20]
Host plants	Tomato, pepper, eggplant, potato, tobacco, dill [20]
Distribution	South and Central America, Asia, USA [20]
Damage	Larvae are defoliators, initially attacking the upper portion of plants and consuming foliage, blossoms and green fruits. They usually consume the entire leaf and are capable of causing high levels of defoliation. The highest foliage consumption occurs during the final instar because of its large size [20]

Figure 8: Tobacco hornworm larva[21]

Life cycle: The larva hatched out of egg has a thick pointed structure. The tomato hornworm has a dark-green horn with black sides, while that of the tobacco hornworm is red. Larvae blend in with the foliage and are not easy detected. They cause considerable damage at the end of the larval period. Adults of both species, emerging out of the pupa, are large moths with stout, narrow wings. [20]

Western corn rootworm

Scientific name	*Diabrotica virgifera virgifera* [22]
Synonym	Billion dollar bug [22]
Order	Coleoptera [22]
Host plants	Rootworm larvae can complete develope only on corn and a few other species of grasses. Studies have shown that rootworm larvae reared on other grasses (specifically yellow foxtail) emerged as adults later and had smaller head capsule size as adults than larvae reared on corn. [22]
Distribution	North America, Europe [22]
Damage	Most of the damage in corn is caused by larval feeding. Newly hatched rootworms locate corn roots in the soil and initially begin feeding on the root. Severe root injury interferes with the roots' ability to transport water and nutrient into the plant which results in reduction of plant growth and reduced grain production. [22]

The Western corn rootworm is one of the most devastating corn rootworm species in North America. A related species, the Northern corn rootworm, D. barberi Smith and Lawrence, co-inhabits in much of the range, and is fairly similar in biology. [22]

Figure 9: Western corn rootworm beetle [23]

Figure 10: Corn rootworm larva [24]

Corn rootworm larvae can destroy significant percentages of corn if left untreated. In the United States, current estimates show that 120 000km² of corn are infested with this pest and area is expected to grow over the next 20 years. The United States Department of Agriculture estimates that corn rootworms cause one billion dollar in lost revenue each year. Therefore, it is also called the "Billion Dollar Bug". [22]

Life cycle: The Western corn rootworm overwinters in the egg stage in the soil. Larvae hatch in late spring time and begin to feed on corn roots. This herbivore goes through three larval instars, pupate in the soil, and emerge as adults in summer. There is one generation per year. After feeding for several weeks, the larvae dig a cell in the soil and molt into the pupal stage. Females mate soon after emergence. Western corn rootworm females need to feed for about two weeks before they can lay eggs. [22]

Biological control: In the USA natural antagonists of the corn rootworm were detected, such as particular ground beetle, predatory flies, parasitic wasps (Braconidae), spiders and hair worms (nematodes). Entomopathogenous nematodes were tested successfully in Hungary, Austria and Italy. A first product due basis of the nematode *Heterorhabditis bacteriophora* is available commercially since 2012. Nematodes can be administered at the moment of sowing or at time of appearance of larvae. At the moment European investigations are conducted on the use of a parasitic fly (Tachinidae), according to the Austrian Agency for Health and Food Safety (AGES). This fly is a natural enemy of the corn rootworm in Mexico. [25]

Spider mites

Scientific name	*Tetranychus urticae* [26]
Synonym	Two-spotted spider mite [26]
Order	Trombidiformes (part of class “Arachnida”) [26]
Host plants	Vegetables, fruit trees, grains, beans, economic crops, ornamental plants [26]
Distribution	Worldwide [26]
Damage	Generally, mites feed on the undersides of leaves. They use their sucking mouthparts to remove plant saps. Infested leaves may turn yellow, dry up and drop in a few weeks. Mites produce large amount of webbing. Plants die when infestation is severe. [26]

Figure 11: Tetranychus urticae [27]

Life cycle: Eggs are laid on the undersides of leaves, often under the webbings, and hatch in 4 or 5 days. Nymph looks similar to the adult. It molts 3 times before becoming an adult. Mites have only 6 legs but they are no insects, they are arachnids. [26]

Whitefly

Scientific name	*Bemisia tabaci* [28]
Synonym	Sweet potato whitefly [28]
Order	Homoptera [28]
Host plants	Vegetables, fruit trees, ornamental plants, weeds [28]
Distribution	Worldwide [28]
Damage	Whiteflies, both the larvae and adults, pierce and suck the sap of the leaves. This causes the weakening and early wilting of the plant resulting in reduced plant growth. Their feeding may also cause yellowing, drying, premature dropping of leaves that result in plant death. Whitefly is the most important carrier of plant viruses that causes diseases of fiber crops, vegetables, fruit trees, and ornamentals. [28]

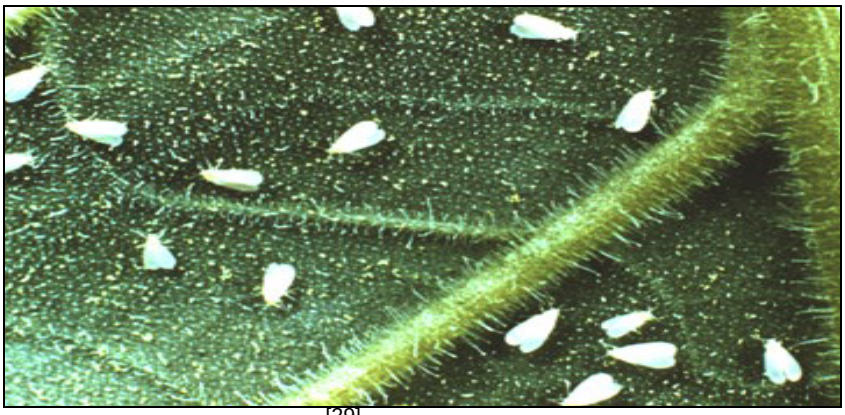


Figure 12: Whiteflies [29]

Other whitefly species:

Bandedwinged whitefly (*Trialeurodes abutilonea*)

Greenhouse whitefly (*Trialeurodes vaporariorum*)

Silverleaf whitefly (*Bemisia argentifolii*)

Spiraling whitefly (*Aleurodicus disperses*) [28]

Scales

Scientific name	*Icerya purchasi* [30]
Synonym	cottony cushion scale [30]
Order	Homoptera [30]
Host plants	Citrus and other fruit trees, banana, cacti, orchids and other tropical ornamentals, palms, figs, grapes, shrubs [30]
Distribution	Worldwide [30]
Damage	Scales remove plant saps by using their sucking mouth parts. Leaves become stunted and turn yellow. Twigs and branches die back and eventually the plant dies when the population level is high. [30]

Figure 13: Cottony cushion scale (Icerya purchase) [31]

2.6. Biological control

Biological control is a method of controlling pests (including insects, mites, weeds and plant diseases) by means of other living organisms. It relies on predation, parasitism, herbivory or other natural mechanisms, but typically also involves an active human management role. It can be an important component of integrated pest management (IPM) programs. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids and pathogens. There are three basic types of biological pest control strategies: importation (sometimes called classical biological control), augmentation and conservation. [32]

Importation or "classical biological control" involves the introduction of a pest's natural enemies to a new location where they do not occur naturally. In many cases the complex of natural enemies associated with a pest may be inadequate. Such situation can occur when a pest is accidentally introduced into a new geographic area, without its associated natural enemies. Classical biological control is long lasting and inexpensive. Other than the initial costs of collection, importation, and rearing, little expense is incurred. When a natural enemy is successfully established it rarely requires additional input and it continues to kill the pest with no direct help from humans and at no cost.

Two examples of successful importation programs are mentioned here:

One of the earliest successes was in controlling *Icerya purchasi*, the cottony cushion scale, which was devastating the California citrus industry in the late 19th century. A predatory insect *Rodolia cardinalis* (the vedalia ladybeetle), and a parasitoid fly were introduced from Australia by Charles Valentine Riley. Within a few years the cottony cushion scale was completely controlled by these introduced natural enemies.

A small wasp, *Trichogramma ostriniae*, was introduced from China to help in controlling the European corn borer (*Ostrinia nubilalis*), one of the most destructive insects in North America. It is a recent example of a long history of classical biological control efforts for this major pest. [32]

A detailed understanding of the biosynthesis and timing of volatile organic compound releases is necessary to use HIPV as a novel tool in crop protection. The perception and exploitation mechanisms of these chemical signals through natural enemies of herbivores also have to be understood for biological control. [33]

2.7. Parasitoids

Parasitoids lay their eggs on or in the body of an insect host, which is then used as a food for developing larvae. The host is ultimately killed. Most insect parasitoids are wasps or flies, and usually have a very narrow host range. [32]

Four of the most important groups are:

- Ichneumonid wasps: They prey mainly on caterpillars of butterflies and moths. [32]

- Braconid wasps: Tiny wasps attack caterpillars and a wide range of other insects including greenfly. They are a common parasite of the cabbage white caterpillar. [32]

- Chalcid wasps: They belong to the smallest insects and parasitize eggs/larvae of greenfly, whitefly, cabbage caterpillars, scale insects and strawberry tortrix moth (*Acleris comariana*). [32]

- Tachinid flies: They parasitize a wide range of insects including caterpillars, adult and larval beetles, true bugs, and others. [32]

Cotesia species

Scientific name	*Cotesia glomerata* [34]
Type	Larva parasitoids [34]
Order	Hymenoptera (Family: Braconidae) [34]
Hosts	Armyworm, bollworm, cabbage looper, cabbageworm, celery looper, corn earworm, cutworm, diamondback moth, gypsy moth, hornworm, stem borer, tobacco budworm, webworm [34]

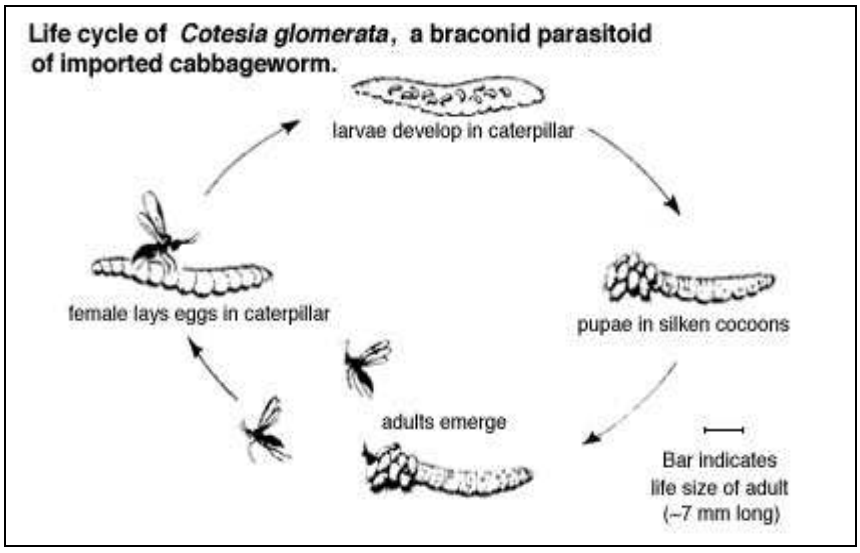


Figure 14: Life cycle of parasitic wasps [35]

Life cycle: Female wasps deposit eggs in bodies of the host by their ovipositor. Eggs increase in size after they are laid. Larvae hatch 2 days later. The first instar parasitoid larvae begin feeding internally after 3-4 days. The immature parasitoids develop through three larval instars in the host body, and then emerge from the host by chewing through the skin. After emergence from the host, larvae of the last instar immediately spin cocoons and pupate. The cocoons are usually found inside host feeding tunnels in leguminous plants. Pupation takes 4-6 days after which adults emerge. Adults are small wasps. They are dark-colored and look like flying ants or tiny flies. After mating the adult female *C. glomerata* look for hosts in leaves and in tunnels of crops. The

life cycle, from egg to adult, is approximately 15-30 days, depending on the species and the temperature. [34]

2.8. Predators

Predators are mainly free-living species that directly consume a large number of prey during their whole lifetime. [32]

Some examples of predators are listed here:

- Ladybugs, and in particular their larvae, are predators of aphids, and also consume mites, scale insects and small caterpillars.
- The larvae of many hoverfly species principally feed upon greenfly. They also eat fruit tree spider mites and small caterpillars. Adults feed on nectar and pollen, which they require for egg production.
- *Phasmarhabditis hermaphrodita* is a microscopic nematode that kills slugs, thereafter feeding and reproducing inside. The nematode is applied by watering onto moist soil, and gives protection for up to six weeks in optimum conditions.
- Other useful garden predators include lacewings, pirate bugs, ground beetles, aphid midge, centipedes, spiders, predatory mites. [32]

Big-eyed bug

Scientific name	*Geocoris spp.* [36]
Order	Hemiptera [36]
Prey insects	Big-eyed bugs are considered an important predator in many agricultural systems and feed on mites, insect eggs and small insects such as pink bollworm, cabbage loopers and whiteflies. [36]

Figure 15: Geocoris punctipes [37]

Big-eyed bugs are true bugs in the order Hemiptera. The two most common species are *Geocoris pallens* and *Geocoris punctipes*. Both are predators and occur in many habitats, including fields, gardens and turf grass. Adult big-eyed bugs have proportionately large eyes. Eggs are deposited singly or in clusters on leaves near potential prey. They develop with incomplete metamorphosis (there is no pupa). It takes approximately 30 days to develop from egg to adult depending on temperature. Both nymphs and adults are predatory, but can survive on nectar when prey insects are rare. Big-eyed bugs, like other true bugs, have piercing-sucking mouthparts and feed by stabbing their prey and sucking the juices. [36]

2.9. Microorganisms

Pathogenic microorganisms include bacteria, fungi, and viruses. They kill or debilitate their host and are relatively host-specific. [32] Microorganisms are mentioned here as herbivore enemies for the sake of completeness. However,

there is not enough evidence that microorganisms can perceive and follow the plant's cry for help.

3. PLANTS' CRY FOR HELP

3.1. Mechanism of indirect defence

In general, inducible defences consist of three steps: surveillance, signal transduction, and the production of defensive chemicals. In the first step, the plant surveillance system detects parasite attacks by specific recognition of signals. The detected signals are then transduced through a network of signal transduction pathways, which eventually lead to the production of defence chemicals. In all cases, induction of plant volatile organic compounds (VOCs) can be triggered by both biotic and abiotic stress. [2]

In induced processes, rather than in the case of constitutive defences, the recognition of the attacking insect and the subsequent signaling of the alarm are preconditions for a fast and efficient defense. Many forms of induced defence are not restricted to local responses at the wounding site, but can be detected systemically throughout the plant. Thus, induced defences also involve the production and accumulation of various VOCs that influence insect attraction/deterrence and inhibit insect growth and development. There are two types of plant inducible defence: direct defences and indirect defences. Chemical substances like toxins, repellents, antifeedants, digestibility reducers and morphological defences such as trichomes, surface waxes and tough foliages belong to direct defences. This type of defence by itself can affect the susceptibility of host plants to insect attacks. Indirect defences include plant traits that by themselves are unable to affect the susceptibility of host plants, but can serve as attractants to natural enemies of the attacking insect. [2]

When plants are attacked, they attract enemies of the herbivores with volatile blends that provide information about the location, activity, number and perhaps even developmental stage of the attacking herbivore. [3] Over 50 different species of plants are reported to produce distinct blends of HIPVs, and these are known to attract a range of herbivore enemies which include predators and parasitoids from five insect orders, furthermore predatory mites, nematodes and birds. [3] Such interactions are largely based on the exceptional capacity of most organisms to associate particular odors with particular rewards. For many predatory, parasitic or pollinating insects, a single associational learning event is

sufficient to link a particular plant volatile with a reward like prey. For other, more specialized insects, the associations can be hard-wired, such as known for some social wasps. In many cases, particular orchids exploit these hard-wired associations. Orchids have evolved deceptive pollination mechanisms. For example, orchids that live deep in conifer forests fool hunting social wasps by releasing a blend of GLVs from their flowers. The wasps mistake the flowers for leaf-feeding caterpillars and, while grappling with the flowers, become part of the pollen postal service when pollinia are attached to their heads or abdomens. The ability to make these olfactory associations is sufficiently valuable that organisms tolerate instances when it is exploited. [3]

Within a single plant species, plant volatile emission can vary with the herbivore present. [3] This variation provides herbivore enemies with valuable information on the identity of prey or host on a plant and their feeding guilds. [3] For example, *Brassica rapa* plants damaged by the root herbivore *Delia radicum* (cabbage rootfly) emit a distinct blend of volatiles from their aboveground tissue that differs significantly from the blend that is released when the plants are attacked aboveground by caterpillars of *Pieris brassicae* (large cabbage white butterfly). 4-Methyltridecane and salicylaldehyde are dominant compounds in the blend of *D. radicum*-damaged plants, whereas methyl salicylate is characteristic for *P. brassicae*-damaged cabbage. When roots and shoots are attacked simultaneously, the GLV hexyl acetate is released in high amounts. [3]

Another work evaluated if corn plants damaged by larvae of the lesser cornstalk borer (*Elasmopalpus lignosellus*) release VOCs which are capable of attracting the egg parasitoid *Trichogramma pretiosum*. The treatments consisted of plants subjected to *E. lignosellus* larvae feeding, plants subjected to mechanical damage and undamaged plants. The total amount of volatiles released did not differ significantly between damaged and undamaged plants. The chemical profile analysis of the extracts from corn plants which were exposed to each of the three treatments also did not show qualitative differences in the compounds identified by GC-MS. However, significant differences were observed in the quantitative analysis when the compounds were evaluated individually. The green leaf volatile (*Z*)-3-hexenyl acetate, the terpenes β-pinene and β-myrcene,

(*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) and benzothiazole were released in significantly greater amounts by plants damaged by larvae than by undamaged plants. In the Y-tube olfactometer, *T. pretiosum* did not show preference for odours from manually damaged plants in comparison to undamaged plants or to undamaged plants contrasted to air. The egg parasitoid preferred odours from plants damaged by larvae in comparison to undamaged plants. These results indicate that *T. pretiosum* can be attracted to the chemical cry for help of corn plants. [33]

Köllner et al.[38] examined maize plants attacked by lepidopteran larvae. The consequence of this herbivory has been the emission of a volatile mixture that consisted mostly of the sesquiterpenes (*E*)-α-bergamotene and (*E*)-β-farnesene. These volatiles are produced by the herbivore induced terpene synthase TPS10 and attracted natural enemies to the damaged plants. A survey of volatiles in maize lines and species of teosinte (*Zea mexicana, Euchlaena mexicana*) showed that the TPS10 products (*E*)-α-bergamotene and (*E*)-β-farnesene are consistently induced by herbivory. This indicates that the release of TPS10 volatiles is a defense trait conserved among maize and its wild relatives. Sequence comparison of TPS10 from maize and its apparent orthologs from four teosinte species demonstrated stabilizing selection on this defense trait. The teosinte volatiles and the enzymatic activity of the apparent TPS10 orthologs were not completely uniform but varied in the ratio of (*E*)-α-bergamotene to (*E*)-β-farnesene products. A single amino acid was identified in the active center which determines the ratio of (*E*)-α-bergamotene to (*E*)-β-farnesene and has changed during the evolution of maize and teosinte species. [38]

The attack of an herbivore results in a large-scale transcriptional rearrangement in the attacked plant. This includes the induction of genes involved in phytohormone biosynthesis and the biosynthesis of secondary metabolites. HIPV blends can comprise tens up to hundreds of components, but only a subset of this complex mixture of components mediates the attraction of carnivorous arthropods. Determining which compounds are involved in the attraction can be done by offering individual compounds, or by interference with

their induction or biosynthesis in the plant. For example, transgenic tobacco plants that are compromised in the emission of certain plant volatiles (terpenoids and green leaf volatiles) are less attractive to a predatory bug [12], and spider mite infested lima bean plants in which a specific step in terpenoid biosynthesis has been blocked are compromised in their attraction of predatory mites during spider mite infestation. [12]

A study of Köpke et al.[39] showed the effect of herbivore oviposition on the transcriptional pattern of plants. *Pinus sylvestris* is known to respond to eggs laid by the sawfly *Diprion pini* on its needles by releasing a blend of terpenoids, including the sesquiterpene (*E*)-β-farnesene. These compounds attract a wasp, *Closterocerus ruforum*, which parasitizes sawfly eggs. *D. pini* oviposition also enhances the transcription of two sesquiterpene synthases, an (*E*)-β-caryophyllene/ α-humulene synthase (PsTPS1) and a 1(10),5-germacradiene-4-ol synthase (PsTPS2). To gain a better understanding of the function of these sesquiterpene synthases in promoting insect egg parasitism, the outcome of *D. pini* oviposition on P. sylvestris has been compared with interaction between other pine and sawfly species: *Neodiprion sertifer* eggs on *P. sylvestris*; *Gilpinia pallida* eggs on *P. sylvestris*, *D. pini* eggs on *Pinus nigra*. The first of these attracts the parasitoid *C. ruforum*, while the latter two do not. PsTPS1 and PsTPS2 transcripts increased significantly only for those species combinations where the odor of egg-laden pine needles was attractive to *C. ruforum*. Moreover enhanced transcription of these genes was found only at those time periods when odour was attractive. Thus the PsTPS1 and PsTPS2 genes are good markers for parasitoid attraction. [39]

Pine species	Sawfly species	Time after egg deposition (day)	Response by egg parasitoids
P. sylvestris	D. pini	2	No attraction
		3	Attraction
		4	No attraction
P. sylvestris	N. sertifer	3	Attraction
P. sylvestris	G. pallida	3	No attraction
P. nigra	D. pini	3	No attraction

Figure 16: Interaction between pine and sawfly species [39]

When *P. sylvestris* twigs were tested with *N. sertifer* eggs, the pattern of response was evident only at third day, but not at second or fourth day after oviposition. The other two combinations (*P. sylvestris* twigs laden with *G. pallida* eggs and *P. nigra* twigs laden with *D. pini* eggs) did not show a significant attraction to *C. ruforum* at any time point tested. The temporal patterns of parasitoid attraction may be a result of when the sawfly egg is most suitable for infestation. [39]

Herbivore-induced emission of plant VOCs is not limited to higher plants. It has been shown that the arsenic hyperaccumulating fern *Pteris vittata* responds to herbivore wounding by emitting the sesquiterpenes (*Z*)-β-farnesene, (*E*)-β-farnesene, (2*Z*, 6*E*)-α-farnesene, (2*E*, 6*E*)-α-farnesene and (*E*)-nerolidol. [2]

Another study investigated whether the emission of VOCs from bracken fern *Pteridium aquilinum* is triggered by herbivory and if so whether it is regulated by the octadecanoid signaling pathway. Feeding of a generalist caterpillar (*Spodoptera littoralis*) and a specialist caterpillar (*Strongylogaster multifasciata*) as well as application of singular and continuous mechanical wounding of fronds induced only very low levels of VOC emission. In contrast, treatment with JA led to the emission of a blend of VOCs that was mainly comprised of terpenoids. Likewise, treatment with the JA precursor 12-oxo-phytodienoic acid (OPDA) and α-linolenic acid also induced VOC emission, but to a lower intensity than the JA treatment. Accumulation of endogenous JA was low in mechanically wounded fronds and these levels were unaffected by the application of oral secretions from generalist or specialist herbivores. The emission of terpenoids upon JA treatment could be blocked with fosmidomycin and mevinolin, which

are inhibitors of the MEP- and MVA- pathways, respectively. These results indicate that similar to higher plants, terpenoid VOCs are produced via these pathways in bracken fern and that these pathways are JA-responsive. However, the very low amounts of terpenoids released after herbivory or mechanical damage are in big contrast to what is known from higher plants. It is speculated that *S. multifasciata* and *S. littoralis* feeding apparently did not induce the threshold levels of JA required for activating the MEP and MVA pathways and the subsequent volatile emission in bracken fern. These plants probably do not need indirect defence because of their effective toxic agents, such as indanones, cyanogen glycosides and tannines were detected amongst others.[40]

Speculations are made about development of indirect defence. On the one hand indirect defence could have its evolutionary origin in formerly direct defence measures. On the other hand indirect defence could have gained more importance in the course of pollinator attraction by volatiles, which is an attribute of flower generating spermatophytes and not of flowerless ferns. [40]

Conclusion: The insect feeding-induced emission of volatiles has been demonstrated for several higher plant species, among others *Brassica rapa*, maize (*Zea mays*), Lima bean (*Phaseolus lunatus*), *Nicotiana attenuata*, and pine (*Pinus sylvestris*), as well as for lower plants like ferns. VOCs represent a phenotypically plastic response to herbivory, which results in changes in interactions between insects and plants.

3.2. Reactions of plants

The role of plants often has been seen as passive in an evolutionary context, and the adaptiveness of crying for help has been challenged. Yet, the evidence that plants benefit in terms of Darwinian fitness increases, and the evolutionary ecological aspects of plants crying for help receive ample interest. [3]

A plant that is attacked has a wide array of potential responses, including various direct and indirect defences. Mounting direct defences, such as toxic secondary metabolites, may be an appropriate first action to stop generalist herbivores. However, many specialist herbivores tolerate or detoxify secondary

metabolites of their host plant. Moreover, specialists may sequester secondary plant metabolites and exploit them in their own defence against carnivores, which is counterproductive to the plant's investments. Therefore, mounting direct defences may not be the most appropriate response to combat specialists. In *Nicotiana attenuata*, when compared to the response to mechanical damage, herbivory by the specialist herbivore *Manduca sexta* results in an attenuated nicotine response and an induced emission of terpenoids that is part of the plant's indirect defence. [13]

By the attack of a herbivore, plant cells will be damaged and, consequently, compounds will unavoidably leak out of the plant, including volatiles. This is similar to what happens when mechanical damage occurs because of, for example, animal moving through the vegetation. However, for a plant, these types of damage can have dramatically different consequences. Mechanical damage is likely to be a discrete event, while a feeding herbivore may continue to feed, to reproduce and thus inflict damage over a longer period of time. Thus, it may pay plants to be able to discriminate between different types of damage and respond differentially. Recent work has shown that WRKY transcription factors may mediate the discrimination between mechanical damage and herbivory by influencing the dynamics of JA titer in the plant. These transcription factors mediate responses in terms of resistance to herbivores and the emission of HIPV with subsequent effects on herbivory intensity. In response to mechanical damage, the plant's first interest may be to close the wound, while herbivory requires more extensive action. The volatiles emitted after a single bout of mechanical damage can attract carnivorous arthropods, but usually this is a short-lasting event. However, when mechanical damage continues in a pattern resembling herbivore feeding, the volatile bouquet includes many compounds that are also induced by herbivory. [13]

Sometimes, it may not pay the plant to emit HIPV. Parasitoids that attack caterpillars usually attack young caterpillars because old caterpillars are much better at defending themselves. For instance, the parasitoid *Cotesia kariyai* attacks caterpillars of the lepidopteran herbivore *Mythimna separata* in the first few instars. Older instars cannot be parasitized as these large caterpillars have

an effective physical defence that may even kill the parasitoid. This parasitoid is not attracted to maize plants infested with fifth or sixth instar larvae, but is attracted to plants infested with first through fourth larval instar caterpillars. The effect can be mimicked by application of regurgitant from third versus sixth instar larvae, indicating that elicitors in regurgitant of the caterpillars are age dependent. [13]

There is further evidence of developmental stage dependent volatile emission. For instance, larvae of *Plagiodera versicolora* (the willow leaf beetle) activate the emission of six out of seventeen detected volatile compounds in young *Salix eriocarpa* (wollypod willow) trees. These six volatile compounds occur in significantly higher amounts after larvae feeding than after adult beetle attack. Larval feeding also results in higher overall emission rates than does adult beetle damage. Egg deposition induces distinct volatile blends and egg-induced volatile blends differ from those induced by larval feeding. [3]

The examination of Baldwin[41] in the Great Basin in Utah showed that wild tabac (*Nicotiana attenuata*) can react to direct or indirect defence according to the herbivore type. Actually *N. attenuata* has an effective direct defence mechanism against insect feeding because of its neurotoxin nicotine. When leaves are damaged by grasshoppers and deltoid moth caterpillars the signal substance JA is generated. JA migrates downwards of the stem into the roots and induces the production of nicotine. The intake of nicotine causes paralysis of muscles. Investigations revealed that a single leaf contains a nicotine dose of eight to ten cigarettes. A few hours after the first insect attack nicotine is transferred from roots and accumulated in the leaves. Herbivores soon stop feeding and disappear from the inedible plant. However caterpillars of *Manduca spp.* are resistant to nicotine. Therefore *N. attenuata* has to change its strategy. Indirect defence has to be activated quickly because each day *Manduca* caterpillars become bigger and more poisonous. They develope a warning coloration (white stripes on green ground and dark eye spots) which signalize their enemies that they have become defendable and inedible due to assimilated nicotine. In this stage the caterpillar is intangible. So tabac has to start its indirect defence early when *Manduca* is small and vulnerable.

N.attenuata recognises *Manduca* at the first attack because of caterpillar saliva. First of all costly nicotine production shuts down and protease inhibitors are generated in leaves which reduce fast growth of *Manduca* caterpillars. Tabac releases volatile cues which attracts *Geocoris* spp. This predatory bug stings into caterpillar and sucks it dry. Tabac is rescued by means of „cry for help“. [41]

Figure 17: Geocoris attack on a young hornworm caterpillar [42]

Figure 18: Manduca sexta caterpillar [18]

A mechanical caterpillar was built in the Max-Planck-Institute in Jena to prove that insect saliva is the essential recognition feature for tabac. When the caterpillar robot („MecWorm“) is feeding on a leaf original caterpillar saliva is dropped into wound. The result was that various volatiles are released due to different saliva types. [41]

Conclusion: Some mixtures of VOCs are produced after mechanical or biological insult, and their composition depends on the mode of damage (single or continuous wounding, herbivore feeding, and egg deposition). The plasticity of volatilome (the VOCs emitting profile of a specific plant) makes these

changes in HIPV blends possible. Plants may also recognize various herbivore enemies due to their oral secretions and response accordingly to a distinct herbivore. When direct defence is useless against a special attacker like *Manduca* larva in the case of wild tobacco, plants can change to the successful strategy of indirect denfence. So plants are able to react very flexible to their surrounding environment and are not only passive individuals.

3.3. Rate of response

Between damage and the induction of a defence response is a time lag which is a major confinement in inducible defences. Herbivore perception, transcriptional responses, protein formation and biosynthetic responses belong to inducible defences. The very first responses to damage can be observed within seconds to minutes, and involve changes in the plasma membrane potential. Subsequent steps involve phytohormonal signaling and transcriptional responses. Damage also results in the emission of volatiles, some of which are not induced but constitutively present, for example, in glands on the leaf surface. Their emission is not under the control of the plant, but a mere consequence of the rupture of cells. GLVs such as C6 alcohols, aldehydes and esters that are produced by the lipoxygenase pathway are usually among the first volatiles to be recorded. Terpenoids occur later. Metabolic changes in plants are usually expressed within hours to days after damage, and these include the emission of HIPVs. Moreover, attraction of carnivorous arthropods to HIPVs is also initiated within hours. These data show that the lag phase between the onset of herbivory and the attraction of natural enemies is in the order of hours. It should be realized that these studies all used an artificial set-up in which the lag phase was assessed as the period since an introduced herbivore started feeding. In nature, many herbivore feeding events are preceded by oviposition. The deposition of eggs can induce volatiles and other changes in plant metabolites. Prior induction can modify later plant responses to other herbivores. It is demonstrated in a modelling study for the tritrophic system *Brassica oleracea-Pieris rapae-Cotesia rubecula* that the lag time between herbivory and the emission of HIPV can be important. [13] The study shows that a lag phase of more than 1 day eliminates the benefit for the parasitoids to respond to HIPV. For *B. oleracea-Pieris brassicae-Cotesia glomerata*

interactions, empirical data show that the attraction of parasitoids is already apparent after 30–60 minutes, and so it seems that the plant response is fast enough to result in selection on parasitoids to use the HIPV. [3]

Conclusion: Although the time lag between herbivory and the expression of the induced defence is a constraint, plants are likely under selection to optimize rather than maximize the response rate. [3] In most cases the rate of volatile emission seems to be high enough to successfully attract the herbivore enemies.

3.4. Plants cry for help: the carnivore perspective

For carnivorous arthropods success in reproduction depends on host or prey location. In search of their herbivorous victim they face a problem because their victim is under natural selection to minimize the emission of cues that can guide the carnivores to them. This problem can be solved by HIPV emitted by the plants from which the herbivores feed: they are emitted in relative large amounts and the composition of the mixture can be herbivore specific. Thus, HIPV are both detectable and reliable cues that can be exploited by carnivorous arthropods. So HIPVs are easier to detect than signals from the arthropod prey or host themselves. A lot of carnivorous species has been demonstrated to be attracted by HIPV, such as parasitoids, predatory mites, bugs and beetles. These mites and insects are usually more sensitive to the volatiles than the analytical equipments. Many carnivore species can effectively learn to respond to or discriminate between HIPV. [13] Thus carnivores can temporarily specialize in responding to certain volatile blends.

In arthropods olfactory perception occurs when volatile compounds bind to olfactory receptors in the antennae, maxillary palps or legs. One or few olfactory receptors are expressed in each olfactory neuron. Neurons containing similar receptors converge in distinct structures called glomeruli in the olfactory bulb or antennal lobe, which are functional units for odorant coding and processing. The number of such glomeruli is variable among insect taxa. [3]

Herbivore enemies may employ olfactory cues to locate the food plants of their prey and hosts by means of two different modes of perception. In one mode,

called “species specific odor recognition”, single compounds that are characteristic of a certain plant species or a group of related species are used for plant recognition. In a second mode, called “ratio-specific odor recognition”, enemies detect a blend of plant volatiles ubiquitous to many families and are able to discriminate differences in ratios among these compounds to find their hosts. [3]

Among herbivore enemies associated with the *Brassicaceae*, species-specific odour recognition might occur in the specialist parasitoid *Diaeretiella rapae*, a braconid wasp that attacks aphids living on Brassicaceae. Electroantennogram (EAG) indicates that *D. rapae* responses to various isothiocyanates and is attracted to them. It was suggested that this parasitoid has specific receptors for isothiocyanates and uses these compounds as cues to locate its hosts. [3]

Ratio-specific odor recognition is maybe dominant when herbivore enemies do not use plant-specific compounds as attractants. EAG and gas chromatography-electroantennographic detection (GC-EAD) have shown that most enemies tested respond to a list of widely spread plant volatiles, such as linalool, DMNT, (*E*, *E*)-α-farnesene, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol and, (*Z*)-3-hexenyl acetate, but to different extents. Therefore, the ability of natural enemies to discriminate among odour blends is not likely to be due to the presence or absence of individual substances, but instead to the differences in the relative proportions of individual constituents. [3]

Ratio-specific odour recognition of a plant is well demonstrated in the case of *Closterocerus ruforum*, an egg parasitoid of the pine sawfly *Diprion pini.* The parasitoid is more attracted to pine foliage on which *D. pini* eggs have been laid than to foliage without eggs. The volatile blend of the egg-laden foliage differs from that of the foliage with no eggs only in an increased amount of (*E*)-β-farnesene. However, this sesquiterpene alone is not attractive. (*E*)-β-Farnesene is attractive when it is present in a mixture with five other terpenes [β-phellandrene, (*E*)- and (*Z*)-β-ocimene, (*E*)-β-caryophyllene and α-humulene], whose amounts are not increased on *D. pini* oviposition. The blend of these five compounds plus (*E*)-β-farnesene was significantly attractive to the parasitoid, but only when the ratios mimicked those emitted from egg-laden pine foliage.

The parasitoid *Cotesia vestalis* is also allured to a mixture of volatiles. In this case, an enemie response requires four herbivore induced volatiles [n-heptanal, α-pinene, sabinene and (*Z*)-3-hexenyl acetate]. These odour compounds are attractive in ratios similar to those emitted by a cabbage plant infested with diamondback moth (*Plutella xylostella*) and presented versus non-infested cabbage. None of the compounds alone is attractive. [3]

A study on the mite *Phytoseiulus persimilis* shows that mixtures are also important to this class of herbivore enemies. Tests were conducted with several major HIPVs of lima bean, like (*E*)- and (*Z*)-β-ocimene, (*Z*)-3-hexenyl acetate, DMNT, TMTT and methyl salicylate, whereas only the last of these compounds was attractive when presented alone. However, a mixture of all five blend components was more attractive than the individual compounds or partial mixtures, when it was compared with odour from lima bean without herbivory. The results suggest that predatory mites perceive plant volatiles not as a mixture of individual compounds, but as a whole blend. [3]

Furthermore, associative learning can adapt parasitoids to alterations of the herbivore-induced volatile blend due to genotype, plant age, and abiotic conditions. For example, females of *Cotesia marginiventris* are also attracted to the full blend of maize volatiles without prior association, indicating that the blend contains additional attractive compounds like the GLVs (*Z*)-3 hexenal, (*E*)-2-hexenal, (*Z*)-3 hexenol, (*Z*)-2-hexenyl acetate, and (*Z*)-3-hexenyl acetate. These volatiles might elicit a positive chemotactic response innate to *C. marginiventris*. [4]

The way in which herbivore enemies exploit plant volatile cues to locate their prey or host depends on their degree of host specificity. For instance, the parasitoid *Cardiochiles nigriceps*, which is specialized on the noctuid *Heliothis virescens* (tobacco budworm), can discriminate between plant volatiles emitted after host feeding versus volatiles emitted after feeding of a non-host, the closely related *Helicoverpa zea* (corn earworm). In contrast, the generalist parasitoid *Campoletis chlorideae* was equally attracted to the volatiles emitted by the feeding of two other noctuid moth larvae, *Helicoverpa armiguera* (cotton bollworm) and *Pseudaletia separata* (oriental armyworm), although the

composition of the two blends was not the same. Among aphid enemies, the specialist parasitoid *Aphidius ervi* recognizes its host *Acyrthosiphon pisum* (pea aphid) over the non-host *Aphis fabae* (bean aphid) on *Vicia faba* (broad bean), whereas a generalist aphid parasitoid *Diaeretiella rapae* did not discriminate between two aphid species based on plant volatiles alone. [3]

Plants attacked by different developmental stages of the same herbivore can release different blends of volatiles. These differences appear to be exploited more by specialists than by generalists. For example, the specialist parasitoid *Cotesia kariayi* was more attracted to maize plants fed upon by early instar larvae of *Pseudaletia separata*, which are suitable for parasitization, than to plants fed upon by late-instar larvae, which are less suitable for parasitization. A similar situation was observed for the specialist predator *Aiolocaria hexaspilota*. By contrast, the generalist parasitoids *Microplitis rufiventris* and *Cotesia glomerata*, which also attack early stages of their hosts, were unable to discriminate between different instars based on plant emitted volatiles. [3]

The volatile blend released from plants can also give valuable information on the number of herbivores that are currently feeding on a plant because the rate of emission of particular compounds is often positively correlated to the amount of inflicted damage. In addition, volatile release can even indicate whether herbivores have already been attacked by parasitoids and the identity of attacking species, which could represent valuable information for other parasitoids. [3]

The next study reveals that volatile blends of damaged plants can contain important information for parasitoids about the physical condition of their herbivorous hosts. The effects of volatiles from cowpea and peabush flowers and *Maruca vitrata* larvae on host selection behaviour of the parasitoid *Apanteles taragamae* were investigated under laboratory conditions by using a Y-tube olfactometer. Naive and oviposition-experienced female wasps were given a choice between several odour sources that included (1) uninfested, (2) *M. vitrata* infested, and (3) mechanically damaged cowpea flowers, as well as (4) stem portions of peabush plants carrying leaves and flowers, (5) healthy *M. vitrata* larvae, (6) live, virus-infected *M. vitrata* larvae and (7) moribund *M. vitrata*

larvae. In all experiments, the responses of the naive and the oviposition-experienced wasps were not significantly different. Therefore, the data for naive and oviposition-experienced wasps were combined. Wasps were significantly attracted to floral volatiles produced by cowpea flowers that had been infested with *M. vitrata* larvae and from which the larvae had been removed. *Apanteles taragamae* females also were attracted to *M. vitrata*-infested flowers after removal of both the larvae and their feces. Female wasps discriminated between volatiles from previously infested flowers and mechanically damaged flowers. Uninfested cowpea flowers attracted only oviposition-experienced wasps that had received a rewarding experience (the parasitization of two *M. vitrata* larvae feeding on cowpea flowers) before the olfactometer test. Wasps also were attracted to uninfested leaves and flowers of peabush. Moreover, they were also attracted to healthy and live virus-infected *M. vitrata* larvae, but not when the latter were moribund. Until now it has been extensively reported for foliar volatiles which can attract carnivorous insects. These data show that flowers of plants also emit parasitoid-attracting volatiles in response to being infested with an herbivore. [43]

Odour sources	Attraction
M. vitrata infested cowpea flowers	+
M. vitrata infested stem portions of peabush plants carrying leaves and flowers	+
M. vitrata infested cowpea flowers, after removal of the larvae	+
M. vitrata infested cowpea flowers, after removal of both the larvae and their feces	+
healthy *M. vitrata* larvae	+
live, virus-infected *M. vitrata* larvae	+
moribund, virus-infected *M. vitrata* larvae	-

Figure 19: Responses of parasitic female wasps to various odor sources

Herbivore enemies presumably benefit from the exploitation of volatile cues attracting them to their preys or hosts. The value of plant volatiles as cues for herbivore enemies may depend on whether enemies respond innately or only after learning. Associative learning of odours is wide spread among both classes of herbivore enemy, parasitoids and predators. Rewarding experiences, such as successful oviposition (parasitoids) or prey capture (predators), in association with plant odours, have a positive impact on foraging behaviour. However, there is accumulating evidence that some enemies also use native responses in association with plant odours. Furthermore, the degree of host specificity affects the effectivity of HIPV exploitation. The examples mentioned demonstrate that the ability of parasitoids to exploit plant volatiles as cues to locate their hosts is higher when host ranges are narrow. [3]

3.5. Plants response within a complex community

HIPV have long been investigated for simple linear tritrophic food chains. However, the emitted HIPVs are available to all community members, and each of them may exploit the volatiles to its own benefit. When volatiles are used in

open communication systems, the emitter has very little control over who the receiver might be and what the information might be used for. Hence, the fitness consequences for a plant with volatile release are rarely straightforward and involve balancing the benefits of the “intended” effects against the costs of the “unintended” effects. For example, the fragrances a plant emits from its flowers to attract pollinators may attract nectar-robbing bees and florivores too. These adverse effects can be fatal for the plant's reproduction. [1]

Privacy in any chemical communication system is highly desired but difficult to obtain when the emitters and receivers do not share the same genome. In the pheromone-mediated, intra-specific sexual signaling that occurs in insects, privacy is more easily attained. In these communication systems, the use of highly specific chemical signals and very sensitive receptors fine-tuned to perceive particular volatile chemical structures makes it hard for predators or competitors to break the code. [1]

The key components in the volatile blends released from herbivore-attacked leaves attract unwanted herbivores in addition to beneficial carnivores. Some herbivorous beetles find their mates by following the green leaf volatiles released from the feeding activities of conspecifics. When GLV-mute plants are planted into native habitats, they are off the radar of certain flea beetles and thus are not colonized. Other lepidopteran herbivores use GLVs as feeding stimulants. When larvae are placed on mute plants, the plants are not recognized as food, and the larvae do not start feeding without the GLV-mediated signal. Some caterpillars use the diurnal rhythms of volatile release, which attract parasitoids, to coordinate their feeding behaviour and to avoid the attracted parasitoids. [1]

HIPVs are usually studied in simple laboratory set-ups such as olfactometers and wind tunnels.[12] Such studies provide information on the potential role of HIPV in plant-carnivore interactions under natural conditions. Several studies have extended these laboratory examinations to semi-field and field set-ups, and compared the results with those from olfactometers and wind tunnels [13]. The limited number of studies that addressed carnivore responses under more natural conditions generally confirm the data from laboratory bioassays. The

conditions under more realistic settings can be more challenging to carnivores. [3]

For instance, the parasitoid *Diadegma semiclausum* is attracted to volatiles from *B. oleracea* plants infested with larvae of diamondback moth (*Plutella xylostella*) in a Y-tube olfactometer. Moreover, uninfested white mustard (*Sinapis alba*) plants also attract this parasitoid. When the parasitoid is exposed to herbivore-infested *B. oleracea* plants in a glasshouse, the parasitoids enter the set-up faster but take more time to find the herbivore-infested plants when white mustard plants are also present than in the absence of white mustard plants. In contrast, the attraction of the predatory mite *P. persimilis* to prey-infested bean plants was not affected by the simultaneous presence of volatiles from non-prey-infested cabbage or cucumber plants, neither in an olfactometer nor in a glasshouse set-up [13].

Under natural circumstances, abiotic factors may affect the production of HIPV and the responses by insects. For instance, UV-B radiation induces JA-mediated plant responses, and UV-B exposure of plants affects the interactions with herbivorous insects as well as their parasitoids. Atmospheric ozone degrades HIPV components such as terpenoids and can reduce the attraction of parasitoids to infested plants. Also, plant-produced compounds emitted by other plants in the environment may affect HIPV-mediated interactions. For instance, the presence of isoprene in the environment, which is emitted by trees such as oaks, willows and poplars, can negatively influence the attraction of parasitoids to HIPV from host-damaged plants.[13]

Plants are members of complex communities, and the infestation of a plant by a single attacker is the exception rather than the rule. There is ample evidence that herbivores may compete through plant-mediated mechanisms such as induced resistance. [13] Moreover, multiple infestation of a plant may also influence the emission of HIPV. Different herbivore species can elicit different signal transduction pathways due to their feeding technique and these pathways may exert cross talk. [3] Generally, leaf chewing herbivores induce only JA signaling, while piercing-sucking herbivores and pathogens tend to induce SA-mediated pathways as well. [2] Most information on cross talk is

available for a negative interaction between the JA and SA signal transduction pathways. A herbivore like the silverleaf whitefly *Bemisia tabaci* that induces SA-dependent defences, represses JA-dependent defences. Thus, herbivores may manipulate plant defences as a decoy strategy, evidence for which is also available for other systems. [3]

An example of multiple herbivory attack is shown for the lima bean (*Phaseolus lunatus*) which is infested by two different herbivores with different feeding techniques, simultaneously. The whitefly (*Bemisia tabaci*) is a phloem-feeding insect, whereas spider mites (*Tetranychus urticae*) are sap-sucking arachnids. The JA signal pathway, induced by *T. urticae*, is inhibited by *B. tabaci attack*. Therefore, whitefly infestation of spider-mite infested plants result in a reduced attraction of predatory mites (*Phytoseiulus persimilis*) compared to attraction to plants infested by spider mites only. This interference follows from the reduction in (*E*)-β-ocimene emission from plants infested by both herbivores. When exogenous salicylic acid (SA) is applicated to mimic *B. tabaci* infestation, similar results in behavioural and chemical analyses were observed. It is shown that, SA and JA signaling pathways can mutually affect each other. SA can suppress JA-dependent defense gene expression, possibly through inhibiting JA synthesis or its action. Similarly, JA has been shown to negatively affect SA-dependent gene expression. In some cases, synergistic effects between the two signaling pathways have been described. Cross-talk between different defence signaling pathways has important consequences for the evolution of plant defence, as it can influence the amount of damage suffered by plants and subsequently influence selection pressure on defence response. [44]

Plant responses to early-season herbivores may extremely alter the phenotype as a result of extensive transcriptional changes. This can have important consequences for interactions with subsequent attackers. [13] Herbivory-induced plant responses can change the associated insect community into one that is more dominated by specialists than by generalists. [3]

Plant volatiles induced during the early phase of attack by egg deposition of herbivorous insects and their consequences on insects of different trophic levels are explored in the next study. In olfactometer and wind tunnel set-ups,

behavioural responses of a specialist cabbage butterfly (*Pieris brassicae*) and two of its parasitic wasps (*Trichogramma brassicae* and *Cotesia glomerata*) to volatiles of a wild crucifer (*Brassica nigra W.D.J. Koch, Brassicaceae*) were investigated. Volatiles were induced by oviposition of the specialist butterfly and an additional generalist moth (*Mamestra brassicae*). Gravid butterflies were repelled by volatiles from plants induced by cabbage white butterfly eggs, probably as means of avoiding competition, whereas both parasitic wasp species were attracted. In contrast, volatiles from plants induced by eggs of the generalist moth did neither repel nor attract any of the tested community members. Analysis of the plant's volatile profile (volatilome) by gas chromatography-mass spectrometry and the structure of the plant-egg interface by scanning with an electron microscopy confirmed that the plant responds differently to egg deposition by the two lepidopteran species. [45]

The enemies of parasitoids (hyperparasitoids), which also occur under natural conditions, have not been investigated until yet. Only little is known about their foraging behaviour. The following study showed that hyperparasitoids find their victims through herbivore-induced plant volatiles emitted in response to attack by caterpillars that in turn had been parasitized by primary parasitoids. Moreover, only one of two species of parasitoids affected HIPV. This inducement of HIPV resulted in the attraction of more hyperparasitoids than volatiles from plants damaged by not parasitized caterpillars. Parasitoids indirectly gave away their presence through their effect on plant odours induced by their caterpillar host. Compounds in the oral secretion of parasitized caterpillars presumably induce these changes in plant volatile emission. These results demonstrate that the effects of HIPV should be placed in a community-wide perspective that includes species in the fourth trophic level too. Furthermore, these findings suggest that the impact of species in the fourth trophic level should also be considered when developing integrated pest management strategies to optimize the use of parasitoids for pest control. [46]

Another study addresses also hyperparasitoids and scrutinize the benefit of plant volatiles. If hyperparasitism is broadly representative in plant-insect food webs at large, it suggests that damage-induced volatiles may not always be

beneficial to plants with major implications for the evolution of anti-herbivore defence and for manipulating plant traits to improve biological control in agricultural crops. [47]

In environment, plants can communicate among themselves by volatile cues. They perceive an attack on neighbour plants due to the chemical cry for help and start priming. Time left after warning is used to mount defence. However, plant defence doesn't start immediately. For example, if insect attack would completely fail to appear, costs for production of volatiles would be wasted and resources would be lacking for growth and generation of seeds. Therefore, plants divide their defence in two steps. First they prepare in their cells production facilities of defence substances. This first step is called priming. The second step is carried out at the real attack. Insect saliva contains the chemical start signal. Production of defence substances start-up at full capacity by compounds in herbivore. [48]

For instance, priming has been observed at lima bean plants. Herbivore-damaged limabeans produce nectar to attract ants and release a chemical cry for help for the attraction of parasitic wasps. In addition to their defence system, limabean plants have a beneficial early-warning system. Volatile cues for parasitic wasps and nectar for ants play an important role in this system. Heil[49] could demonstrate how limabean plants warn each other of an insect attack. He collected a few living herbivorous beetles in a trap to stage an attack. Captured insects fed on limabeans and activated the defence mechanism. A plastic lamination with holes was put over a twig. Below this lamination alarm volatiles were collected. Through a tube, volatiles were transferred to a conspecific plant in a distance from two to three metres. Due to volatile information undamaged limabean starts nectar production and volatile release. After a little while ants appear although there was still nothing to combat. Intact lima bean interprets volatile cues as warning and prepares for a coming pest attack. [49]

Conclusion: Despite all the reports on volatile-mediated attraction of herbivore enemies to damaged plants, its ecological and evolutionary significance for plants is still uncertain. This is because most studies have been carried out under laboratory conditions or with agricultural systems where true evolutionary

inference is not possible. [3] Under laboratory conditions numerous biotic and abiotic factors are absent that affect volatile emission. In natural communities not only beneficial organisms but also further herbivores can be attracted to volatiles, which is adverse for the plant. Multiple herbivory may influence volatile biosynthesis and attraction of herbivore enemies negatively or positively by inhibition of signal transduction pathways or synergistic effects of signal pathways, respectively. Interaction of plants through priming can enhance plant defence which is profitable for plant fitness. Members of the fourth trophic level, hyperparasitoids, may eliminate beneficial organisms of plants and have negative influence on plant fitness. Because of these plenty factors existing in ecological systems it is hard to determine positive impact of volatile emission on plant fitness. However, these results demonstrate that the effects of HIPV should be placed in a community-wide perspective.

3.6. Termination of defence response

Since the emission of HIPV has costs, plants are expected to terminate the emission as soon as carnivores have eliminated the herbivores or have reduced their activity. The application of caterpillar regurgitant to a plant results in the emission of HIPV during several days, so it is likely that after elimination of herbivores, the emission of HIPV continues, albeit at a diminishing rate. Carnivores can also discriminate between HIPV induced by unparasitized and parasitized herbivores. Large-scale physiological changes result from parasitization of caterpillars which is apparent in their regurgitant. When cabbage plants are induced with the regurgitant of parasitized or unparasitized *Pieris* caterpillars, *Cotesia* parasitoids prefer the volatiles from plants treated with regurgitant from unparasitized caterpillars. [13]

There are two interesting modelling studies that address the effects of HIPV dynamics on parasitoid foraging success. These studies show that parasitoids do not benefit from using HIPV when their emission continues for several days after pupation or elimination by predators. From the parasitoid point of view, it is important whether the plant produces the same odour bouquet in response to all herbivore instars or not. This is especially true for parasitoids that can only parasitize a limited subset of the developmental stages of their host.[13]

Conclusion: There is a lack of data on the termination of HIPV emission. Thus, in the future it will be interesting to gain more information about the ending of volatile release by appropriate examinations.

3.7. The use of plant volatiles in biological control

In grass crops like maize and rice, indirect defences have been the subject of much attention because they might offer new strategies for crop protection against herbivores. As synthetic insecticides require high costs and pollute environment, ambition is great to find alternatives. Natural enemies of herbivores show great promise to limit crop damage in an environmentally safe manner if they can be called up in sufficient quantity during herbivore outbreaks.

For example, larvae of the beetle *Diabrotica virgifera virgifera* (western corn rootworm) are an important root pest of maize. This pest causes in the USA annual financial damage amounting to one billion dollar. Thus *D. v. virgifera* is also called „Billion Dollar Beatle“. [50] In response to larvae feeding, maize roots release (*E*)-β-caryophyllene that strongly attracts the entomopathogenic nematode *Heterorhabditis megidis*. However, most North American maize lines do not release the attractive signal from their roots, whereas many European lines and the closest wild relatives of maize, teosinte, do so in response to *D. v. virgifera* attack. Field experiments showed a 5-fold higher nematode infection rate of *D. v. virgifera* larvae on a maize variety with (*E*)-β-caryophyllene release than on a variety without signal release. The (*E*)-β-caryophyllene signal is generated by the (*E*)-β-caryophyllene synthase TPS23, which is independently regulated in leaves and roots in response to damage by different herbivores. Above and below ground, the signal is involved in the defence against herbivores. [4]

The ability of TPS23 to produce (*E*)-β-caryophyllene is widely distributed among the wild relatives of maize and was shown to be under positive selection pressure. However, the loss of (*E*)-β-caryophyllene production in most North American maize varieties is not due to inactive alleles of the TPS23 gene itself, but caused by an alteration of the signal transduction network that abolishes herbivore-induced gene transcription. [4]

Turlings[50] conducted investigations on indirect defence of maize too. He verified with the help of a six-arm olfactometer in labour that entomopathogenic nematodes are attracted to (*E*)-β-caryophyllene of *D. v. virgifera* damaged maize. Nematodes in central pot filled with earth have six options: three tubes lead in pots filled with earth only, other three tubes in pots with maize plants, of which one plant consciously is infested with *D. v. virgifera* larvae. Turlings and his team tried to activate mute genes of maize species. Nevertheless, it is doubtful if application of synthetic pesticides can be reduced drastically by reactivation of indirect defence, as plant defences are not suitable for large fields of monocultures, where the same is cultivated each year. However, plant defence could suffice if well-tried system of crop rotation would be used again in addition. When corn rootworm finds no maize in the next year anymore, but wheat instead, this would harm *D. v. virgifera more than all pesticides. In this way it has been managed that the* „Billion Dollar Beatle“ disappeared *in Switzerland. [50]*

In crop protection, the most important requirements are plants that send out a sufficiently strong and attractive volatile signal after herbivore attack. Among maize species not all genotypes are capable of emitting volatile signals to attract herbivore enemies. Most northern American genotypes have lost the ability to produce (*E*)-β-caryophyllene and thereby lost the defence mechanism against *D. v. virgifera*. The identification of TPS23 gene provides a molecular tool to restore (*E*)-β-caryophyllene production in nonproducing maize lines whereby alternate strategies for *D. v. virgifera* control could be developed in agriculture. The second requirement for the implementation of indirect defence strategies is that a suitable herbivore enemy is present in the region where the crop is grown. This enemy species must be able to control herbivore populations sufficiently to significantly decrease plant damage. Some herbivore enemies were shown to reduce herbivory to plants intensely in field experiments, whereas an interaction between the *large cabbage white butterfly* and the parasitic wasp *Cotesia glomerata* resulted in a higher consumption of leaf material in the parasitized caterpillars. The third requirement is that plant volatiles should not attract additional herbivores. Thereby the effect of an indirect defence will be reduced. In rice, the volatiles emitted after treatment

with JA also attracted females of the herbivore *Nilaparvata lugens* which is an adverse interaction. Last, the indirect defences need to be compatible with other defences of the plant. If the herbivore is subjected to toxins or feeding deterrents formed by direct defences of the plant, it might not represent a good food source or host for herbivore enemies. [4]

Several studies already showed that integrated pest management strategies can manipulate the abundance and distribution of natural enemies successfully. In these push-pull strategies, herbivore enemies are attracted to crops by intercropping with plants that release high levels of volatiles and thus minimize pest problems in an environmentally safe manner. [4]

Alternatively, the manipulation of volatile emission in crop grasses may be a valuable strategy to improve attraction of herbivore enemies. This strategy is maybe aided by engineering of plants that emit strong, readily detectable volatiles that correspond to the preference of a particular enemy species. The development of such plants is now possible due to the recognition of the pathways responsible for the biosynthesis of volatile compounds. However, the effectiveness of these tritrophic interactions needs to be in synergy with the direct defences of the plant to extend the time that herbivores remain vulnerable to attack from foraging enemies. [4]

Defence mechanisms of plants are not suitable for large fields of monocultures, where the same is cultivated each year. Therefore, indirect defence alone is not sufficient to reduce herbivore quantities and minimize the use of synthetic pesticides. Combination of plant defence with well-tried systems like crop rotation or intercropping is needed to control major pest attacks. Genetically modified plants that emit intense, easily detectable volatiles maybe a further strategy to enhance the attraction of herbivore enemies. However, indirect defence needs to be compatible with direct defence to provide plants with high resistance against herbivores. [4]

4. Conclusion

This review has shown that over evolution simple organisms can develop successful behaviour patterns by trying and failing, such as defence measures which increase their chance of survival. The studies mentioned reveal that chemical cry for help is working. Plants' strategy to recruit natural enemies when they are attacked have stood the test. They call the enemies of their enemies when their direct defence such as toxic metabolites is useless. This was observed among other at wild tobacco, maize, rice, limabeans or lower plants like ferns. Chemical signals are widespread in the whole plant kingdom. [51] Plants can perceive their environment and are sensitive to changes. They recognize their enemies with the help of compounds in their oral secretions and choose an appropriate behavioural pattern. Knowledge about HIPVs can help to use them in biological control in agriculture. Crop loss might be reduced by natural enemies of herbivores, which follow plants' chemical cry for help. However, ample information is needed before using indirect defence successfully in biological control. A major improvement in analyzing HIPV emission will come if studies are conducted more in the field to assess how blend composition is affected by the typical biotic and abiotic factors prevailing there. Most examinations on HIPVs have been carried out under the controlled conditions of the laboratory or greenhouse. Though, only in a community context the function of plant volatiles can be understood, for only this context clarifies the fitness value for plants.

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